MORPHOLOGICAL STUDIES OF INDUCED PULMONARY TUMORS IN MICE WITH SPECIAL REFERENCE TO THEIR CYTOGENESIS

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Preface

Histogenesis of pulmonary tumors has long been the subject of many controversies, but no definite conclusion has yet been obtained.

One of the most essential reasons for this seems to be that the normal fine structures of the broncho-alveolar lining tissues could not be clarified by light microscopic observations. In this regard, since 1954, the electron microscopic observations of the lungs of mammals and other vertebrates have been carried out in our laboratory.

Another important reason is that clinically detected lung cancers in men are usually so fully developed that the small lesions of early stage are not available for study. This obstacle can be overcome by employing the modern methods for the rapid induction of pulmonary tumors in mice by potent chemical carcinogens. There are now a number of technics for the successful induction of these tumors in mice. Since Nettleship and Henshaw in 1943 induced pulmonary tumors in mice with ethyl carbamate (urethane), this agent has been frequently used. Because of its water solubility and high tumogenic potency, we adopted this substance as tumogenic agent in our experiments.

I. Materials and Methods

The animals used for these experiments were strain dd inbred adult albino mice.

The mice received intraperitoneal injections of 10 per cent aqueous solution of urethane, once a week for four consecutive weeks. The dose of an injection was 0.01 ml. per gram of mouse, that is, 1 mg. of urethane per gram of mouse.

The animals, 5 males and 5 females, were killed every week after the last

injection up to the 10th week and the lungs were examined grossly for the presence of tumor nodules and, thereafter, processed for the routine histological examinations and the electron microscopic observations.

II. Results

A. Macroscopic Findings

The tumors began to appear macroscopically a few weeks after the last injection. The tumors were always multiple and often situated close to the pleura so that they could be seen upon inspection of the surface. The fully developed tumors were usually sharply circumscribed nodules with a pearly-white color, and they often projected slightly above the pleural surface of the lung.

B. Light Microscopic Findings

In the early stages the small foci of cell proliferations, which are called "hyperplastic foci" by Shimkin, were found along the alveolar walls (Fig. 1). It was shown, by serial sections, that these "hyperplastic foci" were situated completely separate from any possible contact with bronchi or bronchioli.

The fully developed tumors were benign adenomas and most of them presented a uniform picture of closely packed columns of cuboidal or columnar cells. The cellular elements were supported by a sparse stroma of fibrous tissue. The tumors appeared to grow by expansion and no definite fibrous capsule was found.

As in the case of the above mentioned "hyperplastic foci", most of these tumors, not only nodules directly beneath the pleura, but also nodules situated deep in the lung, had no contact with bronchioli (Fig. 2).

Only in the later stages, some of the tumors extended up into close proximity of the bronchioli and in some cases invaded them.

These facts seem to prove conclusively that the induced pulmonary tumors in mice originate in the alveolar region.

C. Electron Microscopic Findings

1. Arrangement and Fine Structure of the Tumor Cells

In the early stages, corresponding with the light microscope findings which defined the "hyperplastic foci", the proliferations of alveolar wall cells were found (Fig. 3).

In the fully developed nodes the tumor cells were arranged partly in acinous patterns and partly in pavement patterns. The cells were cuboidal or columnar in shape and devoid of any malignant sign.

The nuclei were single, round or oval, and the nucleoli were small and did not show any nucleolonema formation, which was often seen in malignant cells.

The mitochondria were rather round or oval than typically rod-shaped. The matrix was generally of higher electron density and sometimes so densely homogeneous that the cristae were indistinguishable.

As to the endoplasmic reticula, most of them were roughly surfaced.

Golgi's complexes were rarely found.

Besides these common organelles, there were found sometimes peculiar structures in the cytoplasm of the tumor cells.

One was lamellar structure in which several mitochondria were encircled by the concentric layers of lamellae (Fig. 4).

Another was spindle-shaped structure which was composed of cytoplasmic bridge and vacuolic halo surrounding the former. The halo had no definite border membrane (Fig. 5).

2. Classification of the Tumor Cells

The tumor cells could be roughly classified into two types according to whether they had osmiophilic bodies in their cytoplasm or not.

The first type of cell with osmiophilic bodies in its cytoplasm resembled the alveolar wall cell in shape (Fig. 6 and 7). Closer observations revealed that among these cells some had many osmiophilic bodies and others rather few of them. As osmiophilic bodies are known to be found characteristically in the alveolar wall cells, we regarded them as one criteria for identifying a cell as an alveolar wall cell or its kin.

The second type of cell, those devoid of osmiophilic bodies in its cytoplasm, were subdivided into ciliated cells and non-ciliated cells.

The ciliated cells (Fig. 8) has cilia and microvilli on their free surface. These cells were similar to the ciliated cells in the normal bronchial epithelium.

The non-ciliated cells (Fig. 9) were devoid of cilia and microvilli, and resembled the cuboidal cells of the terminal bronchioli.

These findings indicate that the cells having characteristics similar to those of epithelial cells in respective parts along the broncho-alveolar system are found simultaneously in the tumor cells.

III. Discussion

The histogenesis of induced pulmonary tumors in mice is a point of controversy. The more prevalent opinion is that the tumors arise from the alveolar region, not from the bronchial region. Our light microscope findings reassure this former opinion. It is likely that in the urethaned mice some underdifferentiated and multipotent cells in the alveolar region are activated by the oncogenic stimuli to give rise to tumor cells.

The normal alveolar wall, as observed with the electron microscope, is covered with two kinds of epithelial cells: alveolar epithelial cells and alveolar wall cells. Ishiko considered that the latter is lower in differentiation as compared with the former according to his comparative morphological studies on lungs. On the other hand, Otsuka showed that in the experimentally produced atelectasis, proliferation of the alveolar wall cells took place. In our experiments, electron microscopic observations revealed that the alveolar wall cells proliferated to form "hyperplastic foci" prior to tumor formation in the urethaned mice.

From the results of our experiments and some references from the literatures we have come now to suspect that the urethane-induced pulmonary tumors in mice might originate from the alveolar wall cells. The pleomorphism of the tumor cells is probably due to the arbitrary differentiation or the metaplasia of the oncoblasts on the process of oncogenesis.

IV. Summary

The strain dd adult mice received intraperitoneal injections of 10 per cent aqueous solution of urethane. Pearly-white tumors began to appear in a few weeks after the last injection.

Light microscopic observations revealed that the benign adenomas arose from the alveolar region remote from any possible contact with the bronchioli.

In the early stages, proliferation of the alveolar wall cells were shown by electron microscopic observations. The tumor cells are divided into three types according to their fine structures; (1) cells bearing osmiophilic bodies in their cytoplasm, which resemble the alveolar wall cells, (2) ciliated cells devoid of osmiophilic bodies, which resemble the ciliated cells of the bronchial epithelium, (3) non-ciliated cells devoid of osmiophilic bodies, which resemble in shape the cuboidal cells of the terminal bronchioli.

Therefore, it is indicated that these induced pulmonary tumors in mice probably originate from the alveolar wall cells.

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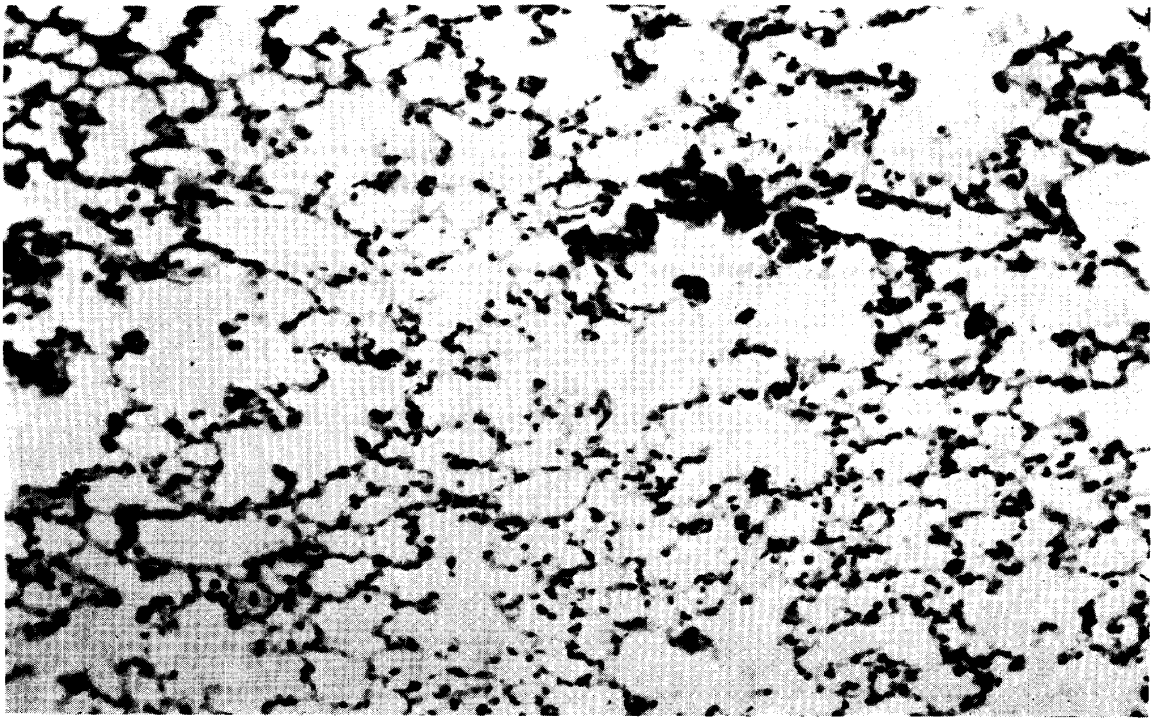

Fig. 1. "Hyperplastic focus".

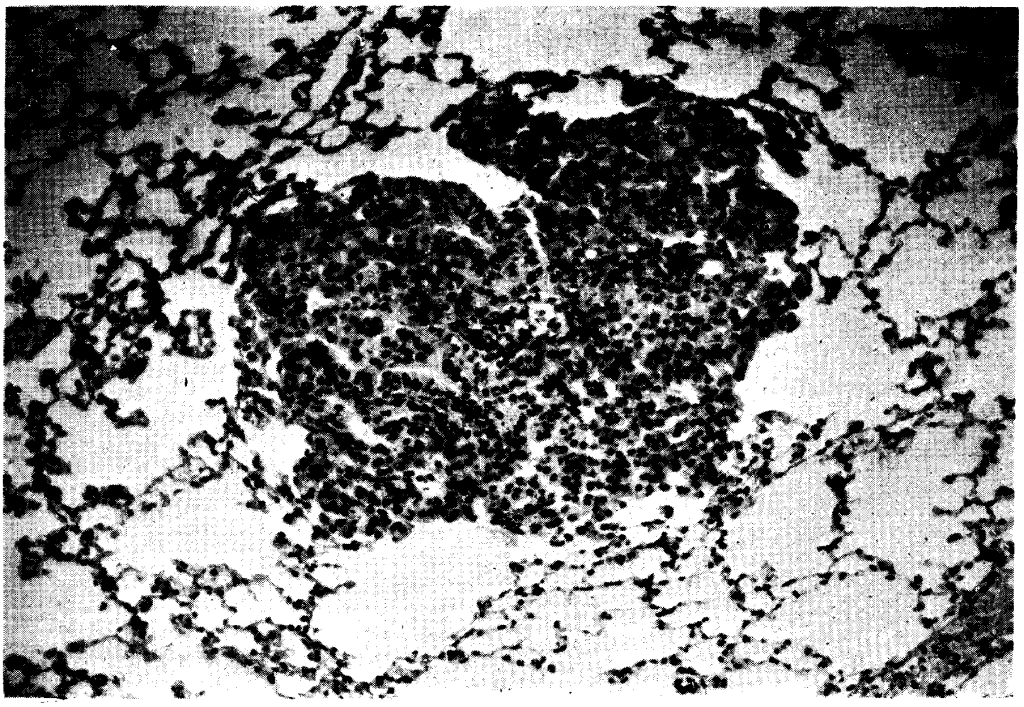

Fig. 2. Fully developed tumor, which does not yet involve any bronchus.

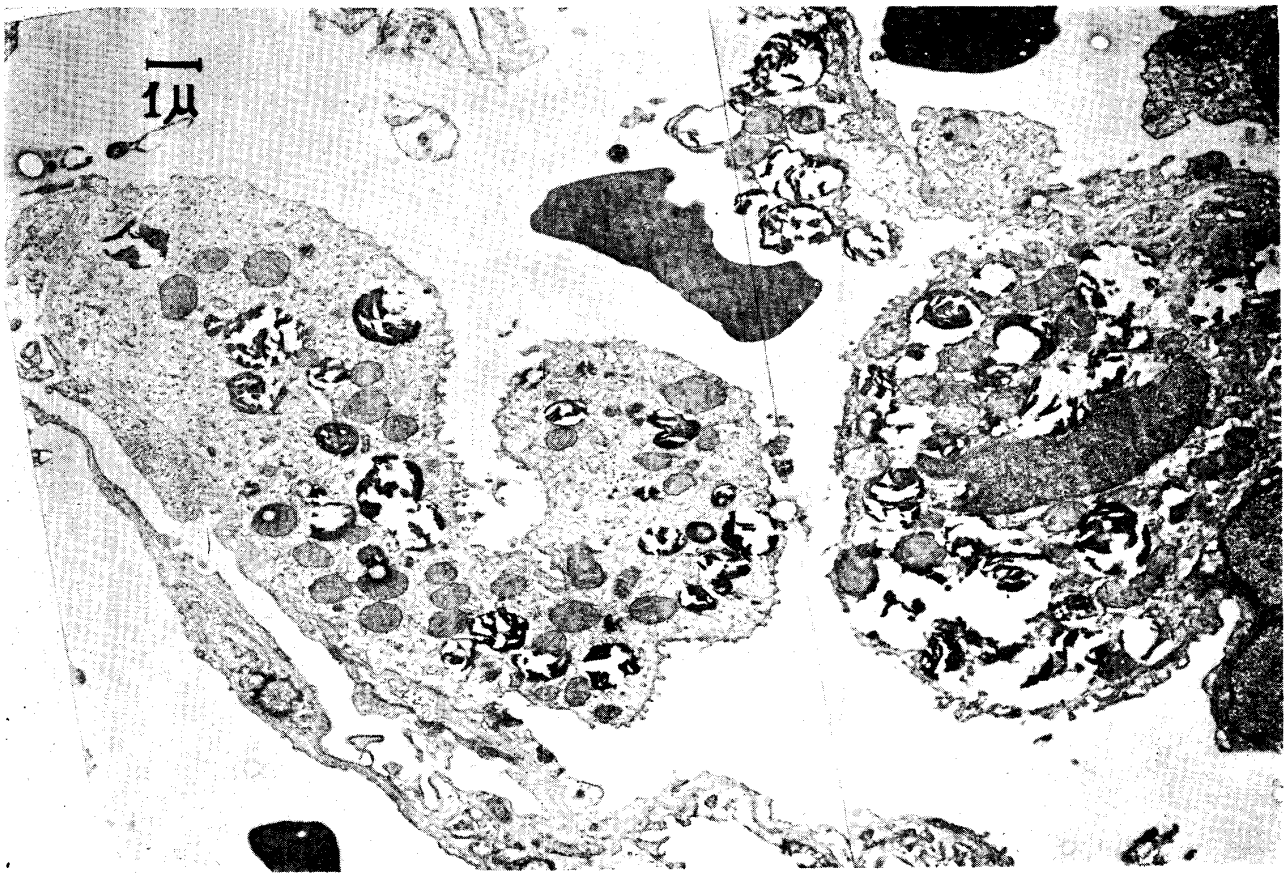

Fig. 3. Proliferation of alveolar wall cells.

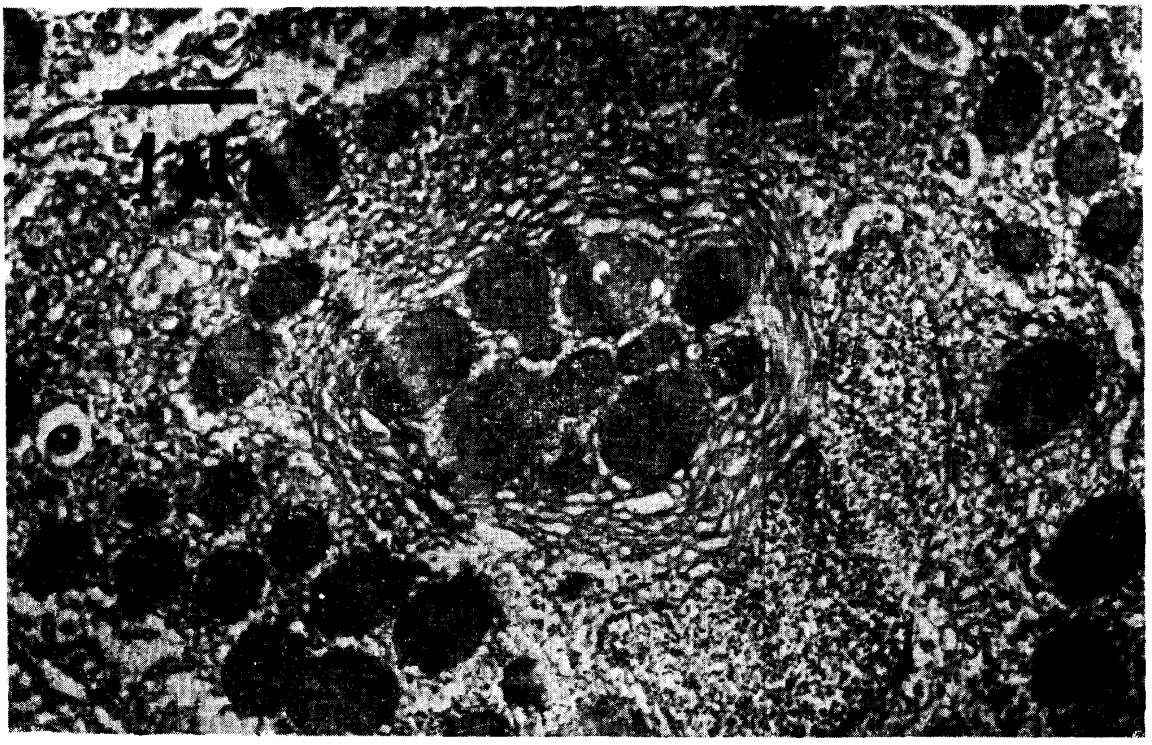

Fig. 4. Lamellar structure surrounding mitochondria, which appears in tumor cell.

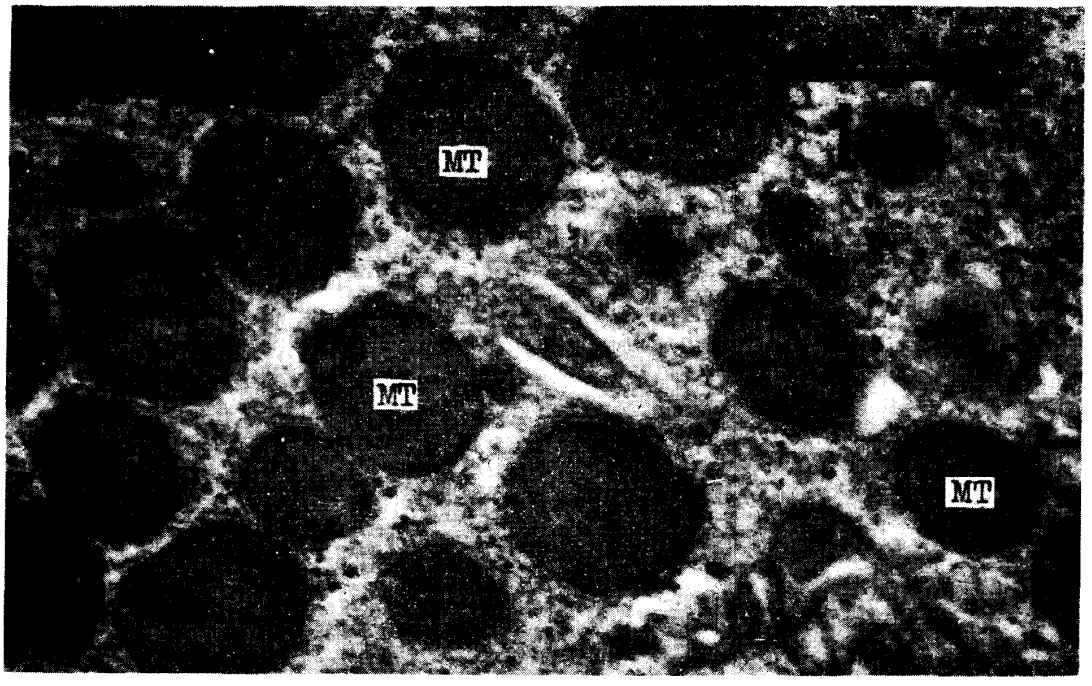

Fig. 5. Spindle-shaped tubal structure appearing in tumor cell.
MT : mitochondria.

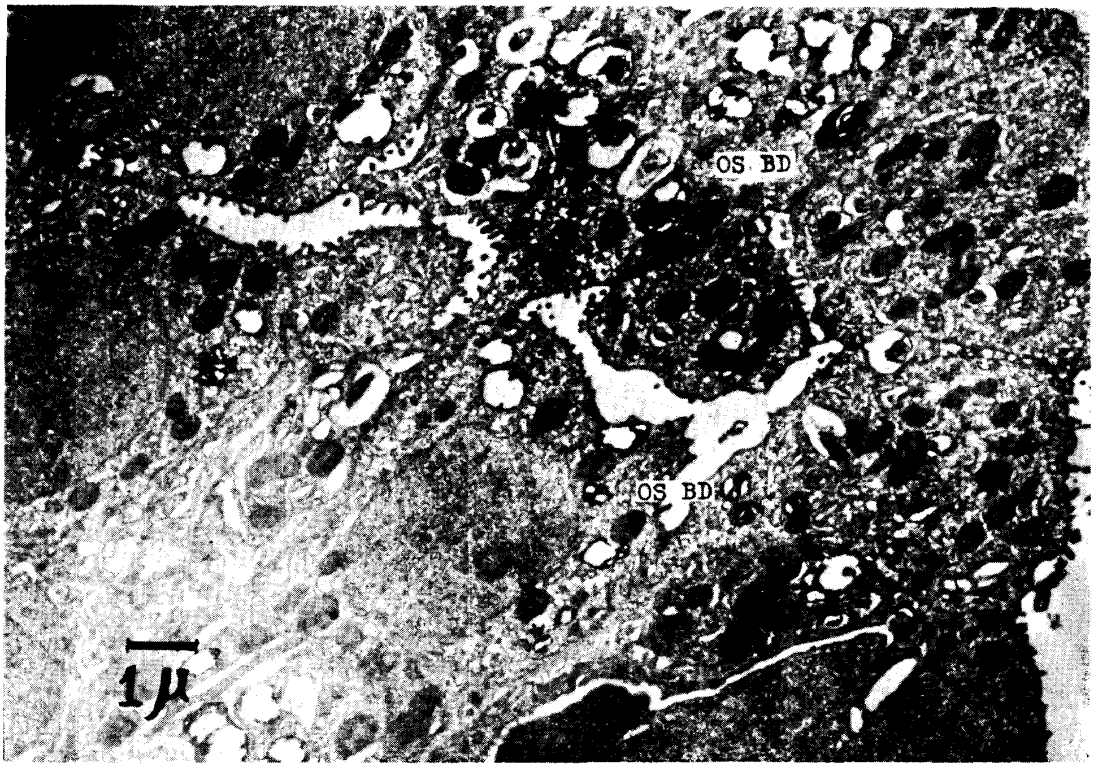

Fig. 6. Tumor cells with osmiophilic bodies in their cytoplasm, whch are arranged in pavement pattern.
OS BD : osmiophilic bodies.

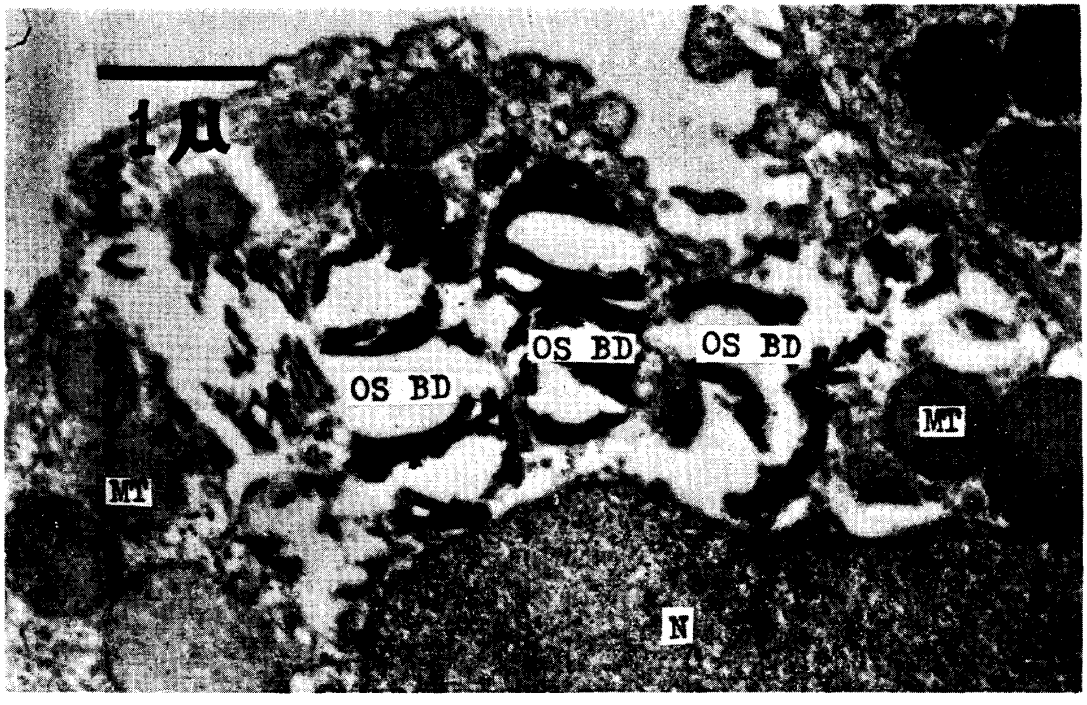

Fig. 7. Osmiophilic bodies in tumor cell.
OS BD : osmiophilic bodies, MT : mitochondria, N : nucleus,

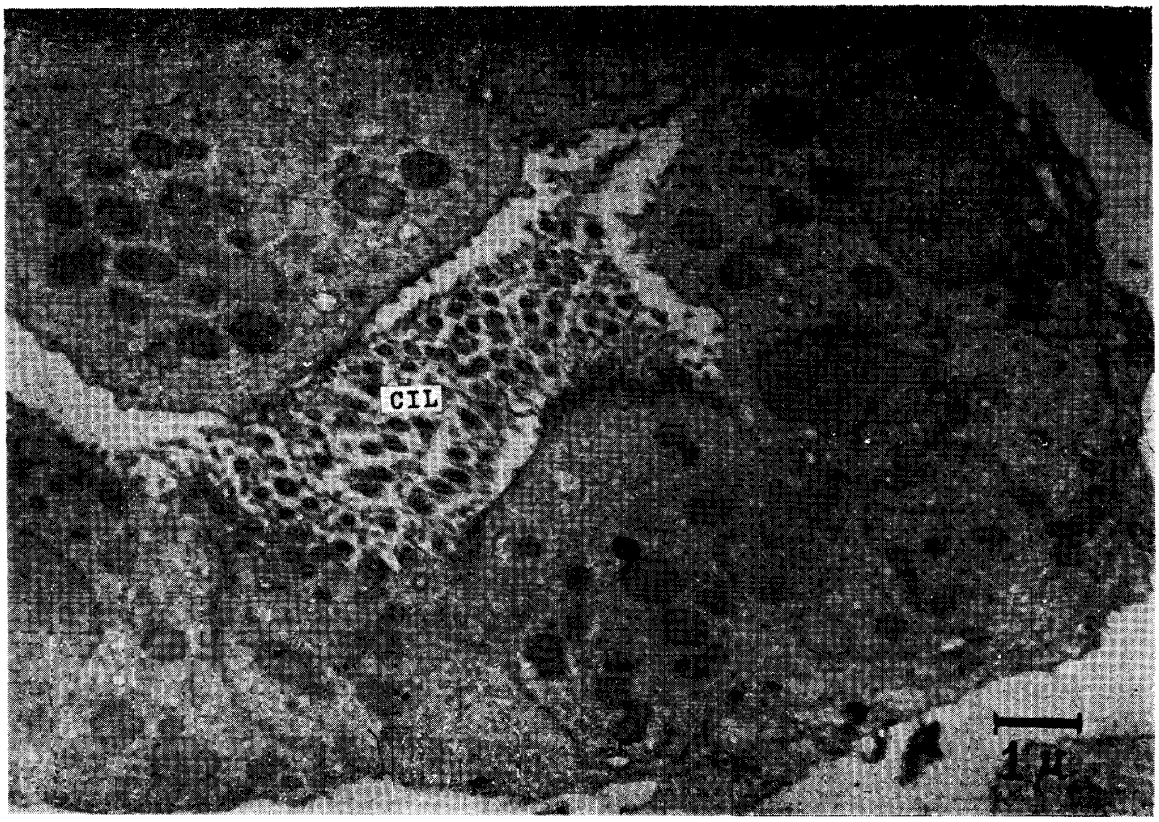

Fig. 8. Ciliated tumor cells.
CIL : cilia.

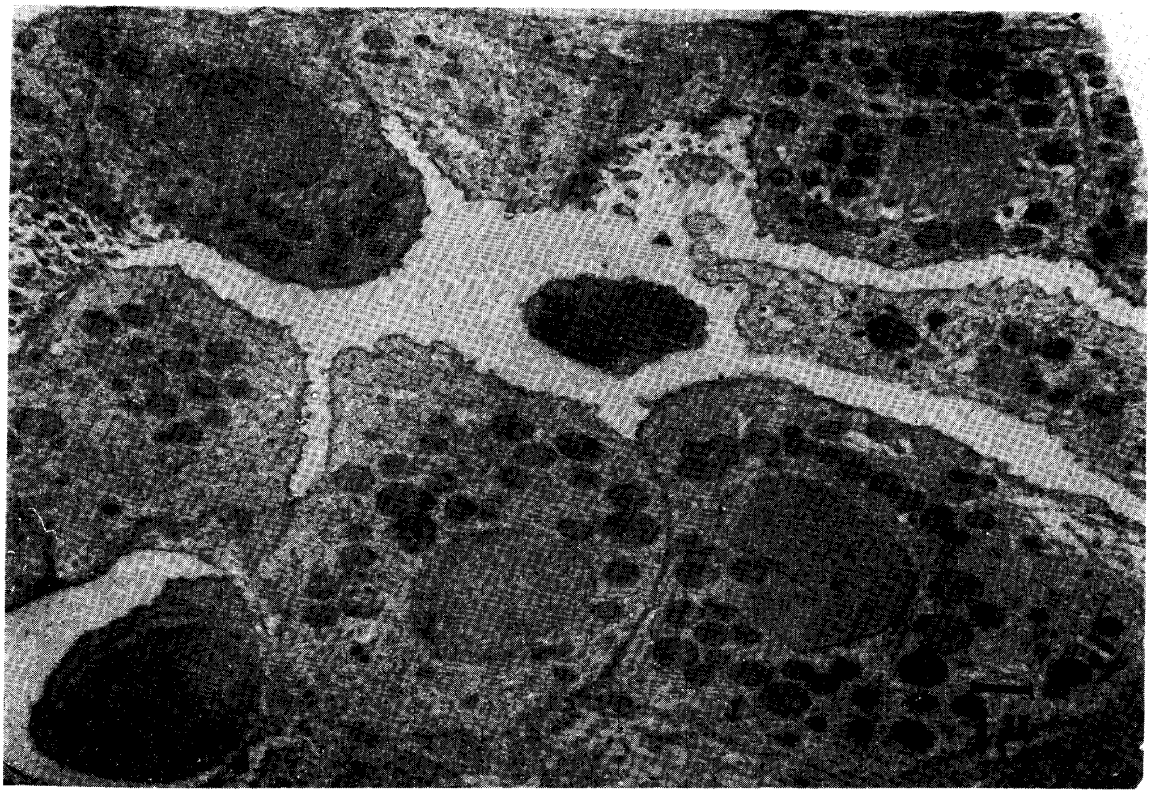

Fig. 9. Non-ciliated tumor cells, which are arranged in acinous pattern.